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HISTOLOGICAL BASIS OF SHANK COLORS IN DOMESTIC FOWL

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BULLETIN 232.

THE HISTOLOGICAL BASIS OF THE DIFFERENT SHANK COLORS IN THE DOMESTIC FOWL.*

By H. R. Barrows.

In this laboratory studies on the inheritance of various characters in poultry have been in progress for some time. Among other characters which have been dealt with from this point of view is the color of the shank (10), (11).† In connection with this work on inheritance, the question arose as to what histological conditions are associated with the different shank colors observed in fowls. As is common knowledge, shank colors, like other characters vary with the breed. Among the shank colors commonly occurring in *Gallus,* and observed in the breeding work here are: white, yellow, blue, black, green, black over green, black over white (dusky), black over yellow (dusky), black over white (dense), black over yellow (dense), black over blue, blue under white, pink, and red. These colors and variations may, in part, appear in individual scales as well as in the shank as a whole. The problem was to determine for each of the above markings the following points:

1. The color of the ultimate pigment granules.
2. Their general nature—whether fatty oils (lipochrome pigments) or granular substance.
3. Their location—In the scales, lower epidermis, dermis, or all three.

Considerable literature on the general histology of the shank of birds is available. Of the earlier writers, Hanau (3) and Jeffries (4) were the first to make exhaustive studies of the dermal structures of birds. The work of the former on the

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† Throughout the paper figures in parentheses refer to the literature list at the end.

corium, and that of the latter on the epidermis is particularly complete. Other writers had, however, made a beginning. Hanau quotes frequently from Leydig (8), whose histological researches included the bird. His contributions extended from 1829 to 1873. Other writers mentioned by Hanau are: Ribbert (14), Müller (9), etc. Krukenberg (7) and Bogdanow (1) investigated feather coloring and incidentally made some observations as to pigmentation of the skin. Kölliker (6) did considerable work on the origin of the pigment cell, and Kerbert (5) on the skin of vertebrates. Of comparatively modern workers, Reichenow (12) on the skin of the legs and feet of birds, and Gadow (2) on their general histology should be noted. While some of these writers, particularly Hanau, dwell to considerable length on pigmentation, none of them correlate the pigmentation with the specific colors which occur in the shanks of the different breeds of poultry. This paper is an attempt to make such correlation.

Methods.

As this study was concerned with the color of the tissues, methods were used which interfered, as little as possible, with the natural condition of the skin. No technique which involved the use of such fat solvents as xylol or absolute alcohol was employed, save for the general histological work. Immediately after dissection from the bird, the material was placed in a weak solution of formalin, about 10%, where it was left until required. Free hand sections were then cut and mounted in liquid glycerine. This method has the advantage of preserving the natural color of the pigment, and also of rendering possible the cutting of sections thick enough to make the dilute oils visible without the presence of a stain. Fat indicators, like Sudan III do not discriminate between oils which are concerned in giving color and those that are not. Nuclear stains render it difficult to decide whether colored particles are pigment granules or nuclear elements. A number of free-hand sections were subjected to various stains, and were found valuable in certain cases, as Sudan III in the study of yellow pigment, and eosin for the location of blood vessels.

For checking previous work on the general histological structure the ordinary paraffin and celloidin methods were em-

ployed. Though many fixing solutions and stains were tried, generally with satisfactory results, the best sections were made by the use of formalin, hematoxylin, eosin, and Sudan III.

The External Structure of the Shank.

A brief review of the structure of the external shank will not be out of place. The outer layer of the epidermis is modified into scales, which vary in size, according to their location on the leg, and the breed of the bird. Two well-defined rows of scales cover the anterior portion in most varieties (2). That row which is located nearest the mid-line usually has the largest scales. These in some cases have a length of one cm. and a breadth of 1.5 cm. Their shape is imperfectly quadrilateral, with the corners slightly rounded. In some birds the arrangement is irregular, and their shape may vary from round to polyhedral. Adjoining the large scales on both sides of the shank are two or three rows of smaller incompletely developed ones, in shape more oval than quadrilateral. On the posterior side we again find two rows similar in size to those on the anterior part. But, as Reichenow (12) has pointed out, "Uebergänge zu den vorigen Formen entstehen, indem die beiden vorderen Reihen von Quertafeln verschmelzen, oder die hinteren in kleine Schilder sich auflösen, oder die kleinen Seitenschilder auch noch zu Quertafeln verwachsen." In the larger scales there is an imperfect adhesion to the under-lying epidermal layer, save at the upper edge and along the sides. The lower edge which is free touches the scale below, and, in the birds studies, over-lapped it for a distance of perhaps a tenth of a centimeter. Jeffries (4), however observed no such over-lapping. The small lateral scales are attached to the under epidermis throughout.

Histology.

The skin of the bird, like that of all vertebrates, consists of two layers; the outer or epidermis, and the inner or corium, cutis, or dermis (2).

THE EPIDERMIS.

A thorough study of epidermal structures, as has been stated, has been made by Jeffries (4). The following statements agree in the main with his observations. The outer portion of the skin of the tarsal region is differentiated into two distinct regions; the rete of Malphigi, and the stratum corneum or horny layer. The latter, which lies outermost, consists of fusiform cells, much flattened, lying in fairly regular rows. Traces of nuclear elements are visible in most specimens, but the outlines of cells are rarely discernible, for the corneum is but a compact mass of cell remnants which has lost the texture of living tissue. This is evidenced by its failure to take protoplasmic stains. It is this layer which is modified to form the scales. As Hanau (3) has noted, the epidermis is invariably thinner than the dermis; it reaches its maximum thickness in the anterior scales of the tarsal region.

The cells of the rete of Malphigi may be divided into three groups according to size and position, although they are of the same origin, springing from the lower layer. All cells of this division are held together by a homogeneous intercellular substance. The basement group comprises but a single layer of columnar cells which are regular in form. It is from these that the other rete as well as the horny layer are derived. This basement stratum lies directly over and in contact with the corium. Immediately above are several layers of polyhedral transition cells, some cells of which project downward between the upper portions of those beneath. A gradual flattening is noted as these transitional cells approach the layer bordering on the corneum. This last group is made up of fusiform cells still more flattened, the outlines of which can be only indistinctly seen. There appear to be no transitional cells between those of the upper Malphigian layer and the corneum. The former resemble the latter in shape but they take protoplasmic stains. No blood vessels or nerves extend into the epidermis.

THE CORIUM.

The histology of this portion of the skin of birds has been thoroughly studied by Hanau (3). Its location is directly under the epidermis. Its structure is that of connective tissue, and

consequently it is not made up of strata corresponding to the rete and corneal layers of the outer skin. The upper portion consists of fine bundles of connective tissue and elastic fibres closely interlaced and running in all directions. Below lies a much looser fabric of larger and more or less horizontal bundles in which fibres predominate. Stained sections show numerous round or oval cells in the upper portions. Below the dermis proper is the subcutaneous connective tissue interspersed with the masses of the panniculus adiposus. The lower and middle corium is richly supplied with blood vessels which give off capillaries. These capillaries penetrate the portions bordering the epidermis, which they appear to touch without penetrating. Nerves also, according to Hanau, are numerous, the fine branches of which in like manner run to the epidermal border without extending beyond. Fatty masses, in varying quantities, are frequently to be found scattered through all parts of the dermis.

Pigmentation.

The various colors seen in the shank are due to the presence of pigment of two kinds; orange-yellow, and brownish-black. (3), (4), (2).

Yellow.—This is a lipochrome pigment, which, when present, is diffused through all parts of the cell; when dilute, it gives a yellow hue; when concentrated, orange. It is found in the epidermis and in the fatty masses of and beneath the corium, and is probably identical with the yellow fat color found in other portions of the body. Various names have been given it. Gadow (2) says, concerning feather color: "Ontochrin, Kühne, der gelbe Dotterfarbstoff aus den Hühnereiern, ist wahrscheinlich identisch mit dem gelben Farbstoffe der Fussbekleidung der Vögel. . . . Krukenberg nennt diesen Stoff Coriosulfurin und hält ihn wie Zoonerythrin und Zooxanthin für ein gefärbetes fettes Oel. Möglicherweise ist es dasselbe wie Zooxanthin."

Black-brown.—The dark color is carried in microscopic pigment granules, which may be scattered through the ordinary cells or be confined to special pigment cells. The former are confined to the epidermis, while the latter may occur in both layers, but infrequently in the epidermis. When granules are present

in the flattened cells of the corneum, they occupy that position which would have been held by the nuclei, had they not degenerated. Instead of being oval groups as are those in the under portions of the Malphigian layer, they lie in short thin lines, which is to be expected, as these cells are greatly compressed. Where these granules occur in the rete layer they tend to cluster around the nuclei, and clearly indicate, by their arrangement, the changes in form which have accompanied these cells in their migration toward the surface.

In the corium, and less frequently in the rete layer, what appear to be dense masses of dark colored pigment granules are found. These are in reality definite cellular bodies, the "verästigte" cells of Hanau (3), densely packed with the black-brown granules. These cells correspond to the "trophoplasts" of Heinke (13) observed in man. There is a central body which sends out branches in all directions. In deeply colored specimens these ramifying strands interlace and form a compact network, which in many cases is so thick as to give the impression of a homogeneous mass; in others only isolated cells are present. Here and there occur round or oval pigmented bodies, which Hanau concluded were the starshaped cells with their pseudopod-like appendages contracted. Pigment cells commonly lie around blood vessels clearly indicating their course. They frequently form a fairly compact tube, but more often are limited to fragments which only partly enclose the vessels. According to Hanau, pigment cells often appear as nerve endings. Pigment cells are to be found in several well-defined localities; in the upper portion of the cutis among the closely interwoven strands of connective tissue, in the region bordering the blood-vessels, in proximity to nerves (3), and surrounding fat masses. Isolated cells are frequently scattered at random through the lower sections of the corium. The lower bodies of pigment play little part in the color of the external shank, as they lie far beneath the opaque connective tissue. Melanin pigment granules are always in the corium contained in pigment cells. When found in the Malphigian layer, pigment cells were of an oval form, no outrunners being observed, however it is probable that other specimens would show them, as their presence is mentioned in this region in the dove in Hanau's monograph. In

size, these cells, when situated in the epidermis occupy a space similar to that filled by several of the Malphigian cells.

Immediately below the epidermis in all specimens examined a space was found a little less in width than that of a row of columnar cells which was practically devoid of pigment. It was impossible to find a single instance in which the pigment cells of the corium penetrated those of the epidermis, although Kerber (15) observed them in the chick embryo.

Zoomelanin is the name given to black-brown pigment in birds by Bogdanow (1) according to Gadow (2).

THE PIGMENT RELATIONS IN THE VARIOUS SHANK COLORS OBSERVED.

White.—Here both lipochrome and melanin pigments are wanting. Gadow (2) observed regarding the coloring of feathers: "Vollkommene Brechung aller eintretenden Lichtstrahlen, ohne Pigment, verursacht weiss," which would apply equally well to the skin though, of course, in the skin one never finds the ivory white of feathers except in the ear-lobes in certain breeds. Melanin pigment may be present in the corium of white shanked birds, either at considerable depths or in quantities insufficient to make itself noticeable. In old birds of yellow shanked breeds, particularly those which have been heavy layers, the yellow of the epidermis frequently disappears and white consequently results. The white shank in this case has a different appearance, however, from that in hereditarily white shanked birds. The thickness of the scales in pure white shanked birds has little to do with the color.

Yellow.—This color results from the presence of zooxanthin in both layers of the epidermis, or in the corneum alone. It is diffused through all parts of the cells and intercellular substances. In brightly colored shanks both epidermal layers exhibit a rich supply of this oil. In young birds the amount in the Malphigian layer is large; as the chick grows this gradually disappears. Old laying hens carry a very small quantity in the horny layer; the rete appearing white to the naked eye. Old hens which for some cause, pathological or other, have never laid possess a deep orange color in both parts of the epidermis. The intensity of the coloration in such cases would seem to indicate that the original supply of lipochrome pigment had not

been used up and also that an additional supply had been deposited. As this fat is present in the scales it is but natural that the intensity of their color should be influenced by their thickness. Consequently when the pigmentation is weak as in old laying hens the scales on the anterior portions of the leg possess the brightest shades. However, in non-laying birds the portion covered by the small lateral scales often appears darker. In this instance the less deeply pigmented thick anterior scales have the effect of diluting the color as a whole. In yellow legged young chicks the pigment is distributed evenly around the shank, since the richly colored Malphigian cells are more influential in determining the resultant color than the incompletely formed and still growing scales.

Blue.—In blue shanked birds zoomelanin is present only in the corium. The blue color is the optical effect resulting when this dark pigment is seen through the semi-translucent Malphigian stratum. As Krukenberg (7) has noted, "Das Blau ist alto hier eine sog. optische Farbe, eine Erscheinung, welche überall da zu Stande kommt, wo das Licht ein trübes Medium durchdringt und von einer schwarzen Unterlage aus alsdann reflectirt wird." As no pigment granules lie in the horny layer the thickness of the scales is scarcely concerned in affecting the depths of color, save where yellow fat occurs. In most of the blue-shanked birds examined the number of pigment cells in the corium was greater than in black legged individuals, and they generally formed a more compact network, however insufficient observations were made to make this conclusion general. The depth of blue color depends directly upon the number of these cells.

Black.—Black shank color results when melanin pigment lies in the epidermis and only under these circumstances, so far as I have observed. It may be heightened by pigment cells in the upper cutis, but pigment in the outer layer is essential to produce the black color. As has been noted two forms of black pigment occur in the epidermis: granules in both layers and pigment cells in the rete. The granules resemble in size the nuclear elements brought out when the tissues are subjected to nuclear stains. In the more deeply colored birds the epidermis is deeply peppered with these particles, the Malphigian layer contains numerous melanin pigment cells, and underneath in

the corium the latter cells lie in an almost unbroken mass. Whenever either kind of pigment was found in the epidermis the corium was also found to be supplied, but there appeared to be no agreement between the quantities in the two layers. The thickness of scales when granules lie in them is of importance in regulating intensity of color. Naturally those scales which are the thickest, everything else being equal, are the darkest.

Lipochrome pigment may be diffused through the epidermis. Where much melanin pigment is present in the epidermis the yellow color of the oil has no effect upon the resultant color, but when the epidermis is sparsely supplied with melanin pigment the black color is modified. This condition will be considered later.

It should be particularly noted that a dense black shank color may be associated with any other underlying dermal or epidermal color. Thus one gets, in different cross-bred birds, as I am informed by Dr. Raymond Pearl, dense black over white, over yellow, over blue, and over green.

Green.—This colored shank is characterized by the presence of lipochrome pigment in the epidermis, and numerous melanin pigment cells in the upper corium. It is an optical color resulting from melanin pigment lying under the semi-transparent yellow epidermis. There is no melanin pigment in the epidermis.

Black over green.—This shank color is similar to that described immediately above with the addition of masses of melanin pigment in the epidermis. In the black portions the melanin pigment occurs as granules in both layers, and often in addition as pigment cells in the Malphigian layer. These black spots seldom cover more than a single scale, and usually are much smaller.

Black over white (dusky white). In this case a thin sprinkling of melanin granules occurs in the epidermal layers, and frequently in addition scattered melanin pigment cells in the Malphigian layer. There are usually melanin pigment cells in the corium but not in sufficient quantity to deepen the shade, else the skin would appear black. There is no lipochrome pigment in either layer.

Black over yellow (dusky yellow). This shank color is similar to that described immediately above with the addition of lipochrome pigment in the epidermal layers.

Black over white (spotted). Here are found black spots in an otherwise white shank. As in the black-over-green color mentioned above, the epidermis in the spotted portion is thickly peppered with black-brown pigment granules in both layers, and frequently supplied with melanin pigment cells in the rete layer. The latter type of pigment is usually found in considerable quantities in that part of the upper corium directly under the spotted region, and to a less degree throughout the corium generally. Lipochrome pigment is lacking.

Black over yellow (spotted). This shank color is due to the same pigmentation as that described immediately above with the addition of orange-yellow pigment in the epidermis.

Black over blue. This type may be identical with the black-over-green color described above with the exception that lipochrome pigment is not present.

Blue under white. This is a white skin mottled with blue blotches or spots. It is nothing more than a white skin and an irregular distribution of melanin pigment cells in the upper dermis.

In all these conditions in which black masses overlie the other colors, the pigment granules are often in much greater numbers than in shanks in which a uniform black color is found. These granules in the epidermis and cells (in the rete layer) are at times so numerous that an intense black color is given the scale without any assistance from the pigment cells in the cutis. Old birds tend to possess these irregular markings more than do young.

Pink.—This color is a modification of white, due probably to an abundance of capillaries in the dermis, and possibly also to thin and unusually transparent scales.

Red.—This color probably results from a congestion of blood in the dermis.

Table showing the nature and location in the skin of the different types of pigment concerned in producing each shank color.

Symbols employed in table: −= absence of pigment specified.
+= presence of pigment specified in very small amounts.
++= presence of pigment specified in average amounts.
+++= presence of pigment specified in very large amounts.
−+= pigment specified may be absent or present in varying but generally small, amounts.

Shank Color.	Epidermis.					Corium.			
	Stratum Corneum.		*Stratum Malphigi.*			*Upper Region.*[1]		*Lower Region.*[2]	
				Melanin Pigment.					
	Lipochrome pigment.	Melanin pigment granules.	Lipochrome pigment.	Granules.	Cells.	Lipochrome Pigment.[3]	Melanin pigment. cells.	Lipochrome pigment.[3]	Melanin pigment cells.[3]
White	−	−	−	−	−	−+	−	−+	−+
Yellow	+ to +++	−	+ to +++	−	−	−+	−	−+	−+
Blue	−	−	−	−	−	−+	++ to +++	−+	−+
Black	−	++ to +++	−	++ to +++	− to +++	−+	− to +++	−+	−+
Black spot on green	+ to +++	++ to +++	+ to +++	++ to +++	− to +++	−+	++ to +++	−+	−+
Dusky white	−	+	−	+	− to +	−+	− to +	−+	−+
Dusky yellow	+ to +++	+	+ to +++	+	− to +	−+	−+	−+	−+
Black spot on white	−	++ to+++	−	++ to +++	− to +++	−+	−to +++	−+	−+
Black spot on yellow	+ to +++	++ to +++	+ to +++	++ to +++	− to +++	−+	− to +++	−+	−+
Black spot on blue	−	++ to +++	−	++ to +++	− to ++	−+	++ to +++	−+	−+
Blue spot on white	−	−	−	−	−	−+	++ to +++	−+	−+
Green	+ to +++	−	+ to +++	−	−	−+	++ to +++	−+	−+

[1]An arbitrary division, comprising approximately the upper fifth of the corium.
[2]The remaining four-fifths of the corium.
[3]Of little or no effect in determining color.

Summary.

1. Yellow and variations are due to the presence of lipochrome pigment in the epidermis, with the absence of melanin pigment.

2. White results from the lack of pigment.

3. Blue color obtains when melanin pigment lies in the upper dermis, with the absence of this type of pigment in the epidermis.

4. Black and variations depend upon the presence of melanin pigment in the epidermis.

5. Green appears when lipochrome pigment lies in the epidermis, and melanin pigment in the corium only.

6. All shades, with the exception of red and pink are the result of various combinations of these pigments; orange-yellow and black-brown.

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Description of Figures.

All figures were drawn at an approximate magnification of 300 diameters. In reproduction they have been reduced by one-fourth. Drawings were made from unstained free-hand vertical sections through the shank skin of the domestic fowl.

Though lipochrome pigment is frequently found in the corium no effort was made to obtain sections which showed it, as its presence has no bearing on the color of the skin and it is difficult to represent it in small quantities in uncolored drawings.

The following characters have the same significance in all of the drawings:

C.—Portion of the corneum.
M.—Rete of Malphigi.
F.—Flattened cells of the rete of Malphigi.
T.—Transition cells of the rete of Malphigi.
B.—Columnar cells of the rete of Malphigi.
D.—A portion of the corium or demis.
G.—Melanin pigment granules.
P.—Melanin pigment cells.
Y.—Lipochrome pigment diffused through cells.
V.—Melanin pigment bordering blood vessels.

Fig. 77. A vertical section of skin from the shank of a white legged hen. Neither melanin nor lipochrome pigment is present.

Fig. 78. A vertical section of skin from the shank of a yellow legged hen. Lipochrome pigment is diffused through the epidermis. Melanin pigment is wanting.

Fig. 79. A vertical section of skin from the shank of a blue legged hen. Melanin pigment cells are thickly scattered through the upper dermis. The epidermis is free from either type of pigment, and there is no lipochrome pigment in the corium.

Fig. 80. A vertical section of skin from the shank of a black legged hen. Melanin pigment granules are scattered thickly through both layers of the epidermis, and melanin pigment cells in the Malphigian layer and through the upper corium. Lipochrome pigment is wanting.

Fig. 81. A similar section from a green shanked bird. The epidermis contains lipochrome pigment in both layers. The corium possesses large numbers of melanin pigment cells in the upper region. Lipochrome pigment is wanting in the dermis, and melanin pigment in the epidermis.

Fig. 82. This drawing shows a portion of a black spot (upper left hand corner) on a green shanked fowl. Lipochrome pigment is diffused through both layers of the epidermis. Melanin pigments granules are numerous in the epidermis of the spotted region, and there are scattered melanin pigment cells in its Malphigian layer. The unspotted portion of the epidermis is free from melanin pigment. Many melanin pigment cells lie beneath the epidermis. There is no yellow pigment in the dermis.

Fig. 83. A vertical section through the skin of a dusky white shank. Here melanin pigment granules are thinly scattered through the epidermal layers. A single melanin pigment cell lies in the rete layer, and similar cells here and there in the corium. There is no yellow pigment in either dermis or epidermis.

Fig. 84. A similar section from a dusky yellow shank. The epidermis contains lipochrome pigment, and melanin pigment granules in small quantities. Melanin pigment cells are sparsely scattered through the Malphigian layer and the dermis.

Fig. 85. A similar section showing a portion of a black spot on a white shank. The spotted region (the upper right hand corner) of the epidermis is thickly supplied with melanin pigment granules in both layers, and sparsely with melanin pigment cells in the rete layer. The latter are present in the corium, in large numbers in the spotted portion. There is no lipochrome pigment.

Fig. 86. A similar section showing a portion of a black spot on a yellow shank. Lipochrome pigment is diffused through both layers of the epidermis. The spotted region of the epidermis (upper left hand corner) is thickly supplied with melanin pigment granules in both layers, and sparsely with melanin pigment cells in the rete layer. The latter cells are present in the upper corium, in large numbers under the spotted portion. Lipochrome pigment is wanting in the dermis.

Fig. 87. A similar section through a part of a black spot on a blue shank. The spotted region of the epidermis (upper left hand corner) is thickly peppered with melanin pigment granules in both layers, and sparsely with melanin pigment cells in the Malphigian layer. The latter cells are numerous in the upper corium. Lipochrome pigment is wanting.

Fig. 88. A vertical section through a blue spot on a white shank. In the spotted region melanin pigment cells lie thickly in the upper corium. Lipochrome pigment is wanting. The epidermis is unpigmented.

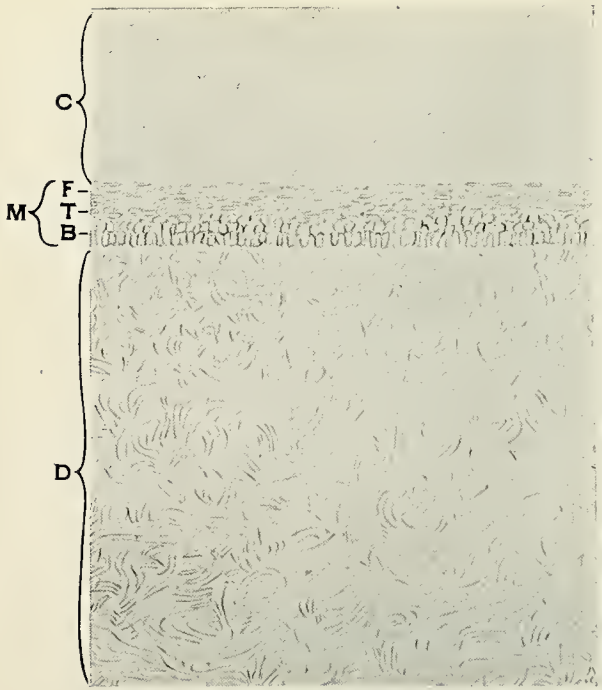

Fig. 77.

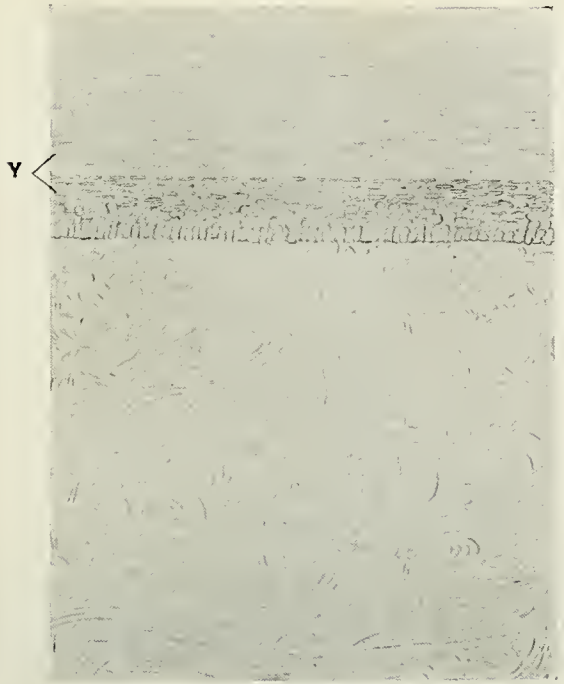

Fig. 78.

Fig. 79.

Fig. 80.

Fig. 81.

Fig. 82.

FIG. 83.

FIG. 84.

FIG. 85.

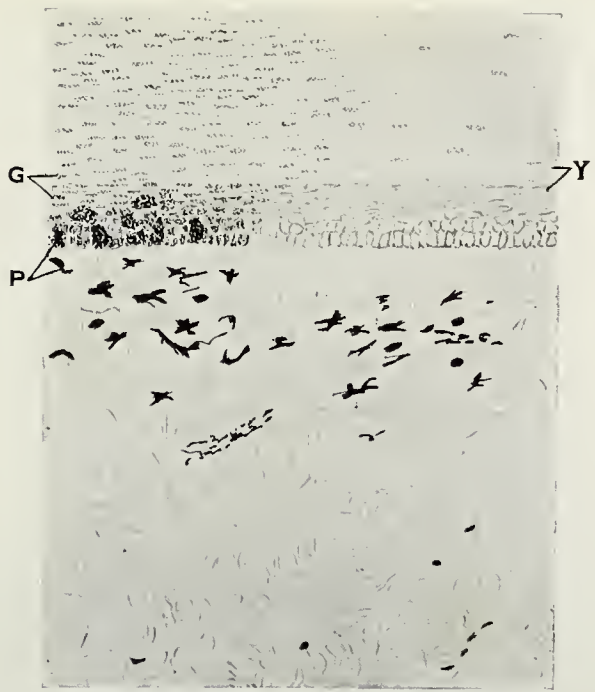

FIG. 86.

FIG. 87.

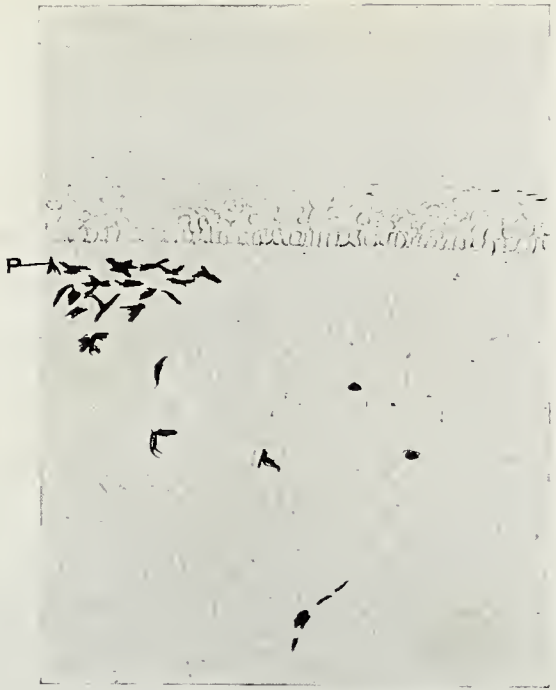

FIG. 88.